

Claims

1. An O⁶-alkylguanine-DNA alkyltransferase (AGT) mutant showing, when compared to the wild type human AGT, two or more advantageous properties selected from
- 5 (a) reduced DNA interaction;
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus;
(c) improved expression yield as soluble protein and improved stability in various hosts;
(d) improved stability under oxidising conditions;
- 10 (e) improved stability within cells after reaction with a substrate;
(f) improved stability outside cells before and after reaction with a substrate;
(g) improved *in vitro* solubility;
(h) improved reactivity against O⁶-alkylguanine substrates;
(i) reduced reactivity against DNA-based substrates; and
- 15 (j) reduced reactivity against N⁹-substituted O⁶-alkylguanine substrates.
2. The AGT mutant according to claim 1 wherein the advantageous properties are
- (c) improved expression yield as soluble protein and improved stability in various hosts and
(h) improved reactivity against O⁶-alkylguanine substrates;
- 20 or
- (c) improved expression yield as soluble protein and improved stability in various hosts,
(d) improved stability under oxidising conditions,
(g) improved *in vitro* solubility, and
(h) improved reactivity against O⁶-alkylguanine substrates;
- 25 or
- (c) improved expression yield as soluble protein and improved stability in various hosts,
(d) improved stability under oxidising conditions,
(f) improved stability outside cells before and after reaction with a substrate,
(g) improved *in vitro* solubility, and
- 30 (h) improved reactivity against O⁶-alkylguanine substrates;
- or
- (a) reduced DNA interaction,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- 35 (c) improved expression yield as soluble protein and improved stability in various hosts,
(h) improved reactivity against O⁶-alkylguanine substrates, and
(i) reduced reactivity against DNA-based substrates;

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or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

5 (c) improved expression yield as soluble protein and improved stability in various hosts,

(e) improved stability within cells after reaction with a substrate,

(h) improved reactivity against O⁶-alkylguanine substrates, and

(i) reduced reactivity against DNA-based substrates;

or

10 (a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

(c) improved expression yield as soluble protein and improved stability in various hosts,

(h) improved reactivity against O⁶-alkylguanine substrates,

15 (i) reduced reactivity against DNA-based substrates, and

(j) reduced reactivity against N⁹-substituted O⁶-alkylguanine substrates;

or

(a) reduced DNA interaction,

(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

20 (c) improved expression yield as soluble protein and improved stability in various hosts,

(e) improved stability within cells after reaction with a substrate,

(h) improved reactivity against O⁶-alkylguanine substrates,

(i) reduced reactivity against DNA-based substrates, and

25 (j) reduced reactivity against N⁹-substituted O⁶-alkylguanine substrates;

3. The AGT mutant according to claim 1 wherein the advantageous properties are

(c') improved expression yield as soluble protein and improved stability in *E. coli* and

(h) improved reactivity against O⁶-alkylguanine substrates;

30 or

(c') improved expression yield as soluble protein and improved stability in *E. coli*,

(d) improved stability under oxidising conditions,

(g) improved *in vitro* solubility, and

(h) improved reactivity against O⁶-alkylguanine substrates;

35 or

(c') improved expression yield as soluble protein and improved stability in *E. coli*,

(d) improved stability under oxidising conditions,

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- (f') improved stability outside cells after reaction with a substrate,
- (g) improved *in vitro* solubility, and
- (h) improved reactivity against O⁶-alkylguanine substrates;
- or
- 5 (a) reduced DNA interaction,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- (c') improved expression yield as soluble protein and improved stability in *E. coli*,
- (h) improved reactivity against O⁶-alkylguanine substrates, and
- 10 (i) reduced reactivity against DNA-based substrates;
- or
- (a) reduced DNA interaction,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- 15 (c') improved expression yield as soluble protein and improved stability in *E. coli*,
- (e) improved stability within cells after reaction with a substrate,
- (h) improved reactivity against O⁶-alkylguanine substrates, and
- (i) reduced reactivity against DNA-based substrates;
- or
- 20 (a) reduced DNA interaction,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- (c') improved expression yield as soluble protein and improved stability in *E. coli*,
- (h) improved reactivity against O⁶-alkylguanine substrates,
- 25 (i) reduced reactivity against DNA-based substrates, and
- (j) reduced reactivity against N⁹-substituted O⁶-alkylguanine substrates;
- or
- (a) reduced DNA interaction,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the
- 30 nucleus,
- (c') improved expression yield as soluble protein and improved stability in *E. coli*,
- (e) improved stability within cells after reaction with a substrate,
- (h) improved reactivity against O⁶-alkylguanine substrates,
- (i) reduced reactivity against DNA-based substrates, and
- 35 (j) reduced reactivity against N⁹-substituted O⁶-alkylguanine substrates;

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4. The AGT mutant according to claim 1 or 2 wherein the advantageous properties are
- (c) more than fivefold expression yield as soluble protein and improved stability in various hosts and
- (h) improved reactivity against O⁶-alkylguanine substrates;
- 5 or
- (c) more than fivefold expression yield as soluble protein and improved stability in various hosts,
- (d) more than fivefold stability under oxidising conditions,
- (g) more than fivefold *in vitro* solubility, and
- 10 (h) more than fivefold reactivity against O⁶-alkylguanine substrates;
- or
- (c) more than fivefold expression yield as soluble protein and improved stability in various hosts,
- (d) more than fivefold stability under oxidising conditions,
- 15 (f) more than fourfold stability outside cells before and after reaction with a substrate,
- (g) more than fivefold *in vitro* solubility, and
- (h) improved reactivity against O⁶-alkylguanine substrates;
- or
- (a) less than 2% of DNA binding,
- 20 (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- (c) more than fivefold expression yield as soluble protein and improved stability in various hosts,
- (h) more than fivefold reactivity against O⁶-alkylguanine substrates, and
- 25 (i) less than 1% reactivity against DNA-based substrates;
- or
- (a) less than 2% of DNA binding,
- (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
- 30 (c) more than fivefold expression yield as soluble protein and improved stability in various hosts,
- (e) more than threefold stability within cells after reaction with a substrate,
- (h) more than fivefold reactivity against O⁶-alkylguanine substrates, and
- (i) less than 1% reactivity against DNA-based substrates;
- 35 or

- (a) less than 2% of DNA binding,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
(c) more than fivefold expression yield as soluble protein and improved stability in various
5 hosts,
(h) more than fivefold reactivity against O⁶-alkylguanine substrates,
(i) less than 1% reactivity against DNA-based substrates, and
(j) less than 2% reactivity against N⁹-substituted O⁶-alkylguanine substrates;
or
10 (a) less than 2% of DNA binding,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
(c) more than fivefold expression yield as soluble protein and improved stability in various
hosts,
15 (e) more than threefold stability within cells after reaction with a substrate,
(h) more than fivefold reactivity against O⁶-alkylguanine substrates,
(i) less than 1% reactivity against DNA-based substrates, and
(j) less than 2% reactivity against N⁹-substituted O⁶-alkylguanine substrates;
- 20 5. The AGT mutant according to claim 1 or 3 wherein the advantageous properties are
(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli* and
(h) improved reactivity against O⁶-alkylguanine substrates;
or
(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,
25 (d) more than fivefold stability under oxidising conditions,
(g) more than fivefold *in vitro* solubility, and
(h) more than fivefold reactivity against O⁶-alkylguanine substrates;
or
(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,
30 (d) more than fivefold stability under oxidising conditions,
(f') more than fourfold stability outside cells after reaction with a substrate,
(g) more than fivefold *in vitro* solubility, and
(h) improved reactivity against O⁶-alkylguanine substrates;
or
35 (a) less than 2% of DNA binding,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,

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- (c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,
(h) more than fivefold reactivity against O⁶-alkylguanine substrates, and
(i) less than 1% reactivity against DNA-based substrates;
or
- 5 (a) less than 2% of DNA binding,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,
(e) more than threefold stability within cells after reaction with a substrate,
- 10 (h) more than fivefold reactivity against O⁶-alkylguanine substrates, and
(i) less than 1% reactivity against DNA-based substrates;
or
(a) less than 2% of DNA binding,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the
- 15 nucleus,
(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,
(h) more than fivefold reactivity against O⁶-alkylguanine substrates,
(i) less than 1% reactivity against DNA-based substrates, and
(j) less than 2% reactivity against N⁹-substituted O⁶-alkylguanine substrates;
- 20 or
(a) less than 2% of DNA binding,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
(c') more than fivefold expression yield as soluble protein and improved stability in *E. coli*,
- 25 (e) more than threefold stability within cells after reaction with a substrate,
(h) more than fivefold reactivity against O⁶-alkylguanine substrates,
(i) less than 1% reactivity against DNA-based substrates, and
(j) less than 2% reactivity against N⁹-substituted O⁶-alkylguanine substrates;
- 30 6. The AGT mutant according to claim 1 or 2 wherein the advantageous properties are
(c) more than tenfold expression yield as soluble protein and improved stability in various hosts,
(d) more than tenfold stability under oxidising conditions,
(f) more than sixfold stability outside cells before and after reaction with a substrate,
- 35 (g) more than tenfold *in vitro* solubility, and
(h) more than tenfold reactivity against O⁶-alkylguanine substrates;
or

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- (a) no detectable DNA binding,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
(c) more than tenfold expression yield as soluble protein and improved stability in various
5 hosts,
(e) more than sixfold stability within cells after reaction with a substrate,
(h) more than tenfold reactivity against O⁶-alkylguanine substrates, and
(i) no detectable reactivity against DNA-based substrates;
or
10 (a) no detectable DNA binding,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
(c) more than tenfold expression yield as soluble protein and improved stability in various hosts,
15 (e) more than sixfold stability within cells after reaction with a substrate,
(h) more than tenfold reactivity against O⁶-alkylguanine substrates,
(i) no detectable reactivity against DNA-based substrates, and
(j) no detectable reactivity against N⁹-substituted O⁶-alkylguanine substrates;
or
20 (a) reduced DNA interaction,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
(c) more than tenfold expression yield as soluble protein and improved stability in various hosts,
25 (d) more than tenfold stability under oxidising conditions,
(e) more than sixfold stability within cells after reaction with a substrate,
(f) more than sixfold stability outside cells before and after reaction with a substrate,
(g) more than tenfold *in vitro* solubility,
(h) more than tenfold reactivity against O⁶-alkylguanine substrates, and
30 (i) no detectable reactivity against DNA-based substrates;
or
(a) reduced DNA interaction,
(b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
35 (c) more than tenfold expression yield as soluble protein and improved stability in various hosts,
(d) more than tenfold stability under oxidising conditions,

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- (e) more than sixfold stability within cells after reaction with a substrate,
 - (f) more than sixfold stability outside cells before and after reaction with a substrate,
 - (g) more than tenfold *in vitro* solubility,
 - (h) more than tenfold reactivity against O⁶-alkylguanine substrates,
 - 5 (i) no detectable reactivity against DNA-based substrates, and
 - (j) no detectable reactivity against N⁹-substituted O⁶-alkylguanine substrates.
7. The AGT mutant according to claim 1 or 3 wherein the advantageous properties are
- (c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,
 - 10 (d) more than tenfold stability under oxidising conditions,
 - (f') more than sixfold stability outside cells after reaction with a substrate,
 - (g) more than tenfold *in vitro* solubility, and
 - (h) more than tenfold reactivity against O⁶-alkylguanine substrates;
- or
- 15 (a) no detectable DNA binding,
 - (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
 - (c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,
 - (e) more than sixfold stability within cells after reaction with a substrate,
 - 20 (h) more than tenfold reactivity against O⁶-alkylguanine substrates, and
 - (i) no detectable reactivity against DNA-based substrates;
- or
- (a) no detectable DNA binding,
 - (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the
 - 25 nucleus,
 - (c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,
 - (e) more than sixfold stability within cells after reaction with a substrate,
 - (h) more than tenfold reactivity against O⁶-alkylguanine substrates,
 - (i) no detectable reactivity against DNA-based substrates, and
 - 30 (j) no detectable reactivity against N⁹-substituted O⁶-alkylguanine substrates;
- or
- (a) reduced DNA interaction,
 - (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the
 - nucleus,
 - 35 (c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,
 - (d) more than tenfold stability under oxidising conditions,
 - (e) more than sixfold stability within cells after reaction with a substrate,

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- (f') more than sixfold stability outside cells after reaction with a substrate,
 - (g) more than tenfold *in vitro* solubility,
 - (h) more than tenfold reactivity against O⁶-alkylguanine substrates, and
 - (i) no detectable reactivity against DNA-based substrates;
 - 5 or
 - (a) reduced DNA interaction,
 - (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus,
 - (c') more than tenfold expression yield as soluble protein and improved stability in *E. coli*,
 - 10 (d) more than tenfold stability under oxidising conditions,
 - (e) more than sixfold stability within cells after reaction with a substrate,
 - (f') more than sixfold stability outside cells after reaction with a substrate,
 - (g) more than tenfold *in vitro* solubility,
 - (h) more than tenfold reactivity against O⁶-alkylguanine substrates,
 - 15 (i) no detectable reactivity against DNA-based substrates, and
 - (j) no detectable reactivity against N⁹-substituted O⁶-alkylguanine substrates.
8. The AGT mutant according to any of claims 1 to 7 wherein between 1 and 25 amino acids of the wild type human AGT are substituted by other amino acids, and optionally 1 to 5 amino acid out of the continuous chain at one, two or three positions are deleted or added and/or 1 to 4 amino acids at the N-terminus or 1 to 40 amino acids at the C-terminus are deleted.
- 20 9. The AGT mutant according to claim 8 wherein two or more modifications are selected from
- 25 (A) Cys62 replacement by Ala or Val;
 - (B) Gln115-Gln116 replacement by Ala-Asn, Asn-Asn, Ser-His, Ser-Ser, Pro-Pro, Pro-Ser, Pro-Thr, or Thr-Ser;
 - (D) Gly131-Gly132 / Met134-Arg135 replacement by Val-His / Leu-Arg, Lys-Thr / Leu-Ser, Gln-Val / Leu-Ser, or Met-Thr / Met-Val, or Gly131-Gly132 / Met134 replacement by Val-His /
 - 30 Leu;
 - (E) Cys150-Ser151-Ser152 replacement by Asn-Ile-Asn, Pro-Leu-Pro, Pro-Arg-Thr, Ser-Phe-Pro-, or Ser-His-Thr-, or Cys150-Ser151 replacement by Phe-Asn or Arg-Asn, or Cys150 / Ser152 replacement by His / Thr, Leu / Asn, Leu / Asn, Leu / Pro or Pro / Leu, or Cys 150 replacement by Ser or Thr;
 - 35 (F) Pro140 / Asn157 / Ser159 replacement by Phe / Arg / Glu, or Pro140 / Asn157 / Gly160 replacement by Met / Trp / Val, or Asn157 / Ser159-Gly160 replacement by Gly / Glu-Ala, Gly

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/ Asn-Trp, Pro / Gln-Cys or Gly-Gln-Trp, or Asn157 / Ser159 replacement by Gly / Glu, or Asn157 replacement by Gly or Arg; and

(G) truncation after Gly182;

and optionally 1 to 10 additional amino acid modifications.

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10. The AGT mutant according to claim 8 wherein three or more modifications are selected from

(A) Cys62 replacement by Ala or Val;

(B) Gln115-Gln116 replacement by Ala-Asn, Asn-Asn, Ser-His, Ser-Ser, Pro-Pro, Pro-Ser,

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Pro-Thr, or Thr-Ser;

(C) Lys125 replacement by Ala and Ala127-Arg128 replaced by Thr-Ala;

(D) Gly131-Gly132 / Met134-Arg135 replacement by Val-His / Leu-Arg, Lys-Thr / Leu-Ser,

Gln-Val / Leu-Ser, or Met-Thr / Met-Val, or Gly131-Gly132 / Met134 replacement by Val-His / Leu;

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(E) Cys150-Ser151-Ser152 replacement by Asn-Ile-Asn, Pro-Leu-Pro, Pro-Arg-Thr, Ser-Phe-Pro-, or Ser-His-Thr-, or Cys150-Ser151 replacement by Phe-Asn or Arg-Asn, or Cys150 / Ser152 replacement by His / Thr, Leu / Asn, Leu / Asn, Leu / Pro or Pro / Leu, or Cys 150 replacement by Ser or Thr;

(F) Pro140 / Asn157 / Ser159 replacement by Phe / Arg / Glu, or Pro140 / Asn157 / Gly160

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replacement by Met / Trp / Val, or Asn157 / Ser159-Gly160 replacement by Gly / Glu-Ala, Gly / Asn-Trp, Pro / Gln-Cys or Gly-Gln-Trp, or Asn157 / Ser159 replacement by Gly / Glu, or Asn157 replacement by Gly or Arg; and

(G) truncation after Gly182;

and optionally 1 to 10 additional amino acid modifications.

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11. The AGT mutant according to claim 8 wherein two or more modifications are selected from

(A) Cys62 replacement by Ala;

(B) Gln115-Gln116 replacement by Ser-His;

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(D) Gly131-Gly132 / Met134-Arg135 replacement by Lys-Thr / Leu-Ser, or Gly131-Gly132 / Met134 replacement by Val-His / Leu;

(E) Cys150-Ser151-Ser152 replacement by Asn-Ile-Asn, or Cys 150 replacement by Ser or Thr;

(F) or Asn157 / Ser159 replacement by Gly / Glu; and

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(G) truncation after Gly182;

and optionally 1 to 10 additional amino acid modifications.

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12. The AGT mutant according to claim 8 wherein three or more modifications are selected from
- (A) Cys62 replacement by Ala;
 - (B) Gln115-Gln116 replacement by Ser-His;
 - 5 (C) Lys125 replacement by Ala and Ala127-Arg128 replaced by Thr-Ala;
 - (D) Gly131-Gly132 / Met134-Arg135 replacement by Lys-Thr / Leu-Ser, or Gly131-Gly132 / Met134 replacement by Val-His / Leu;
 - (E) Cys150-Ser151-Ser152 replacement by Asn-Ile-Asn, or Cys 150 replacement by Ser or Thr;
 - 10 (F) or Asn157 / Ser159 replacement by Gly / Glu; and
 - (G) truncation after Gly182;
- and optionally 1 to 10 additional amino acid modifications.

13. The AGT mutant according to claim 8 wherein three or more modifications are selected from
- (A) Cys62 replacement by Ala;
 - (B) Gln115-Gln116 replacement by Ser-His;
 - (C) Lys125 replacement by Ala and Ala127-Arg128 replaced by Thr-Ala;
 - (E) Cys150-Ser151-Ser152 replacement by Asn-Ile-Asn, or Cys 150 replacement by Ser or Thr;
 - 20 (F) or Asn157 / Ser159 replacement by Gly / Glu; and
 - (G) truncation after Gly182;
- and optionally 1 to 10 additional amino acid modifications.

- 25 14. The AGT mutant according to claim 8 selected from mutants with modifications

Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Asn157Gly, Ser159Glu, truncated after Gly182;

- 30 Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Asn157Gly, Ser159Glu;

Gln115Ser, Gln116His, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu; and

- 35 Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182.

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15. The AGT mutant according to claim 8 with modifications Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182.
- 5 16. The AGT mutant according to claim 8 with modifications Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally 1 to 10 additional amino acid modifications.
- 10 17. The AGT mutant according to claim 16 with modifications Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally 3 to 7 additional amino acid modifications.
- 15 18. The AGT mutant according to claim 16 with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Ser, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from Gln115Ser, Gln116His;
Ser150Asn, Ser151Ile, Ser152Asn;
20 Lys8Thr, Lys32Ile, Leu33Phe, Thr127Ala, Ser150Asp, Ser151Gly, Ala154Thr;
Lys32Ile, Leu33Phe, Ser150Val, Ser152Arg, Gly153Asp, Ala154Asp;
Lys32Ile, Leu33Phe, Ser150Gly, Ser151Gly, Ser152Asp, Ala154Asp;
Ser150Val, Ala154Asp;
Ser150Glu, Ser151Gly, Ser152Glu, Ala154Arg;
25 Lys8Thr, Thr127Ala, Ala154Thr;
Lys32Ile, Leu33Phe;
Ala154Thr;
Leu33Phe;
Ser151Gly;
30 Ser150Asp;
Thr127Ala; and
Lys32Ile, Leu33Phe, and deletion of Leu34
19. The AGT mutant according to claim 8 with modifications Cys62Ala, Gln115Ser,
35 Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally 1 to 10 additional amino acid modifications.

20. The AGT mutant according to claim 19 with modifications Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Asn, Ser151Ile, Ser152Asn, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally 3 to 7 additional amino acid
5 modifications.

21. The AGT mutant according to claim 8 with modifications Cys62Ala, Gln115Ser, Gln116His, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Ser, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from
10 Gln115Ser, Gln116His;
Ser150Asn, Ser151Ile, Ser152Asn;
Lys8Thr, Lys32Ile, Leu33Phe, Thr127Ala, Ser150Asp, Ser151Gly, Ala154Thr;
Lys32Ile, Leu33Phe, Ser150Val, Ser152Arg, Gly153Asp, Ala154Asp;
Lys32Ile, Leu33Phe, Ser150Gly, Ser151Gly, Ser152Asp, Ala154Asp;
15 Ser150Val, Ala154Asp;
Ser150Glu, Ser151Gly, Ser152Glu, Ala154Arg;
Lys8Thr, Thr127Ala, Ala154Thr;
Lys32Ile, Leu33Phe;
Ala154Thr;
20 Leu33Phe;
Ser151Gly;
Ser150Asp;
Thr127Ala; and
Lys32Ile, Leu33Phe, and deletion of Leu34.

22. The AGT mutant according to claim 8 with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Ser, Asn157Gly, Ser159Glu, truncated after Gly182.

23. The AGT mutant according to claim 8 with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser, Cys150Ser, Asn157Gly, Ser159Glu, truncated after Gly182.
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24. The AGT mutant according to claim 8 with modifications Lys32Ile, Leu33Phe, Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Gly, Ser151Gly, Ser152Asp, Ala154Asp, Asn157Gly, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from
35 Gln115Ser, Gln116His;

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Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and
deletion of Leu34.

25. The AGT mutant according to claim 8 with modifications Lys32Ile, Leu33Phe, Cys62Ala,
5 Lys125Ala, Ala127Thr, Arg128Ala, Cys150Val, Ser152Arg, Gly153Asp, Ala154Asp,
Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from
Gln115Ser, Gln116His;
Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and
deletion of Leu34.

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26. The AGT mutant according to claim 8 with modifications Lys32Ile, Leu33Phe, Cys62Ala,
Lys125Ala, Ala127Thr, Arg128Ala, Cys150Asn, Ser151Ile, Ser152Asn, Ala154Thr,
Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from
Gln115Ser, Gln116His;
15 Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and
deletion of Leu34.

27. The AGT mutant according to claim 8 with modifications Lys32Ile, Leu33Phe, Cys62Ala,
Lys125Ala, Ala127Thr, Arg128Ala, Cys150Ser, Ala154Thr, Asn157Gly, Ser159Glu,
20 truncated after Gly182 and optionally further mutations selected from
Gln115Ser, Gln116His;
Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and
deletion of Leu34.

28. The AGT mutant according to claim 8 with modifications Cys62Ala, Lys125Ala,
Ala127Thr, Arg128Ala, Cys150Val, Ser152Arg, Gly153Asp, Ala154Asp, Asn157Gly,
Ser159Glu, truncated after Gly182 and optionally further mutations selected from
Gln115Ser, Gln116His;
Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and
30 deletion of Leu34.

29. The AGT mutant according to claim 8 with modifications Cys62Ala, Lys125Ala,
Ala127Thr, Arg128Ala, Cys150Gly, Ser151Gly, Ser152Asp, Ala154Asp, Asn157Gly,
Ser159Glu, truncated after Gly182 and optionally further mutations selected from
35 Gln115Ser, Gln116His;
Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and
deletion of Leu34.

30. The AGT mutant according to claim 8 with modifications Cys62Ala, Lys125Ala, Ala127Thr, Arg128Ala, Cys150Glu, Ser151Gly, Ser152Glu, Ala154Arg, Asn157Gly, Ser159Glu, truncated after Gly182 and optionally further mutations selected from
- 5 Gln115Ser, Gln116His;
Gly131Lys, Gly132Thr, Met134Leu, Arg135Ser; and
deletion of Leu34.
31. A method for detecting and/or manipulating a protein of interest wherein the protein of
- 10 interest is incorporated into a fusion protein with an AGT mutant according to anyone of
claims 1 to 30 the AGT fusion protein is contacted with particular AGT substrates carrying a
label, and the AGT fusion protein is detected and optionally further manipulated using the
label in a system designed for recognising and/or handling the label.
32. The method according to claim 31 wherein an AGT fusion protein mixture containing the
AGT fusion protein of the protein of interest and the AGT mutant and a further AGT fusion
protein is contacted with a particular substrate, for which either the AGT mutant or the further
AGT is selective, the mixture is treated with a further substrate, and the AGT fusion protein of
the protein of interest and the AGT mutant is detected and optionally further manipulated
- 20 using the label in a system designed for recognising and/or handling the label.
33. The method according to claim 32 wherein the further substrate is added to the AGT
fusion protein mixture after complete reaction of the mixture with the particular substrate.
34. The method according to claim 32 wherein the further substrate is added to the AGT
fusion protein mixture together with the particular substrate.
35. The method according to claim 34 wherein, in the system designed for recognising
and/or handling the label, the label of the particular substrate interacts with the label of the
- 30 further substrate.
36. The method according to claim 35 wherein the label of the particular substrate and the
label of the further substrate are compounds of a fluorescence resonance energy transfer
pair (FRET) or one fluorophore and one quencher for a proximity assay.
- 35
37. An AGT fusion protein comprising an AGT mutant according to anyone of claims 1 to 30
and a protein of interest.